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Abstract: To estimate the geographical extent of introgression, we studied the genetic structure of sympatric and allopatric populations of hybridizing freshwater snail species Viviparus ater and V. contectus in central Europe. Six allozyme loci which were variable in Lake Garda, Italy in a previous study (five nearly diagnostic loci between the two species and one highly polymorphic locus in V. contectus) were analyzed from ten sympatric locations and four allopatric populations each for the two species. Presumably introgressed genes (low allele frequencies) were found from at least one locus in seven out of the ten sympatric sites. These seven sites covered most of northern Italy. The data indicate that introgression has occurred from Viviparus contectus to V. ater and vice versa. Therefore, there is a possibility of widespread introgression or mosaic zones in nature. However, we cannot rule out that the observed patterns are due to the shared ancestry. V. ater possessed low genetic variation (the jackknifed mean of Wright's FST±S.E. over four loci was 0.041±0.004). On the other hand, V. contectus showed high genetic differentiation (the jackknifed mean of FST± S.E. over six loci was 0.546±0.166). Although introgression may have caused evolutionary changes in V. ater and V. contectus, it was not strong enough to level out the genetic differences between the two species, which may have originated from isolation among populations in V. contectus and a past bottleneck event in V. ater

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GENETIC VARIATION IN SYMPATRIC AND ALLOPATRIC POPULATIONS OF HYBRIDIZING FRESHWATER SNAIL SPECIES (*VIVIPARUS ATER* AND *V. CONTECTUS*)

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ABSTRACT

To estimate the geographical extent of introgression, we studied the genetic structure of sympatric and allopatric populations of hybridizing freshwater snail species *Viviparus ater* and *V. contectus* in central Europe. Six allozyme loci which were variable in Lake Garda, Italy in a previous study (five nearly diagnostic loci between the two species and one highly polymorphic locus in *V. contectus*) were analyzed from ten sympatric locations and four allopatric populations each for the two species. Presumably introgressed genes (low allele frequencies) were found from at least one locus in seven out of the ten sympatric sites. These seven sites covered most of northern Italy. The data indicate that introgression has occurred from *Viviparus contectus* to *V. ater* and vice versa. Therefore, there is a possibility of widespread introgression or mosaic zones in nature. However, we cannot rule out that the observed patterns are due to the shared ancestry. *V. ater* possessed low genetic variation (the jackknifed mean of Wright's $F_{ST} \pm$ S.E. over four loci was 0.041 ± 0.004). On the other hand, *V. contectus* showed high genetic differentiation (the jackknifed mean of $F_{ST} \pm$ S.E. over six loci was 0.546 ± 0.166). Although introgression may have caused evolutionary changes in *V. ater* and *V. contectus*, it was not strong enough to level out the genetic differences between the two species, which may have originated from isolation among populations in *V. contectus* and a past bottleneck event in *V. ater*.

INTRODUCTION

Hybridization and introgression are at the centre of evolutionary interest because they can help to understand how reproductive isolation evolves. Examples of hybridization and introgression have been reported from many plant (Rieseberg & Wendel, 1993) and animal species (Harrison, 1993) including molluscs (Woodruff & Gould, 1987; McDonald, Seed & Koehn, 1991; Falniowski, Kozik & Szarowska, 1993; Porter & Ribi, 1994; Katoh & Ribi, 1996; Yokogawa, 1996). Natural hybridization takes place in narrow area of contact zones or mosaic zones (Rand & Harrison, 1989). Through introgression, species may acquire genetic polymorphism (Echelle & Connor, 1989). Hybridization and introgression between species may affect evolutionary events including speciation and phylogenetic relationships.

First generation hybrids between the freshwater snails *Viviparus ater* and *V. contectus* were collected in nature and their hybrid status confirmed with allozyme markers (Katoh & Ribi, 1996). The estimated frequency of the F_1 hybrids was 0.74% in Lake Garda, Italy (Katoh & Ribi, 1996). Allozyme data from Lake Garda are consistent with the hypothesis of gene introgression from *V. ater* into *V. contectus* (Porter & Ribi, 1994) and vice versa (Katoh & Ribi, 1996). Crossing experiments revealed a Mendelian hybrid system with one pair of linked loci (*GPI* and *PNP*; Katoh & Ribi, 1996). *V. ater* and *V. contectus* co-occur at high densities in man-made structures such as harbours and canals. Their dispersal is limited because of brooding. The purpose of this study is to estimate the extent of introgression of allozymes based on allele frequency data.

MATERIALS AND METHODS

A total of 890 freshwater snails was collected from ten sympatric locations and four allopatric populations each, of *Viviparus ater* (499 snails) and *V. contectus* (391 snails) in Italy, Switzerland, and Germany between October 1992 and August 1994 (Fig. 1 and Table 1). Each allopatric population was coded by a combination of a number which represents a specific site and a lower case letter which indicates an exist-

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ing species. Sympatric sites were coded by a number only. If both species were present at a sampling site or in a lake where a sampling site is located, we treated the site as sympatric in this study. Only *V. ater* was collected at the sympatric site Magadino, Lake Maggiore. Lake Chiem, Germany (Population 1c) belongs to the Danube drainage system. Lake Constance (2a) is part of the Rhine system. The remaining sites are in the Po plain. Snails were transported alive to the University of Züurich-Irchel, Switzerland and frozen at −75°C until analyzed electrophoretically.

Standard horizontal starch-gel electrophoresis was performed. Procedures for tissue-extract preparation, and electrophoresis were similar to those of Murphy, Sites, Buth & Haufler (1990) with minor modifications. Five allozyme loci were nearly diagnostic between the two species in Lake Garda, Italy in the previous study (Katoh & Ribi, 1996). One locus [*PGDH*] was highly polymorphic in *V. contectus*. These six allozyme loci (β-galactosidase, EC 3.2.1.22, *βGAL*; glucose-6-phosphate isomerase, EC 5.3.1.9, *GPI*; malate dehydrogenase-1, EC 1.1.1.37, *MDH-1*; phosphoglucomutase, EC 5.4.2.2, *PGM*; phosphogluconate dehydrogenase, EC 1.1.1.44, *PGDH*; purine-nucleoside phosphorylase, EC 2.4.2.1, *PNP*) were stained for the newly collected 890 snails. We used a single gel buffer system of 'JRP' following Avise, Smith & Ayala (1975) for the six allozyme loci studied. Alleles which were most abundant at each locus of *V. ater* in Lake Garda were designated *100*, and the other alleles were named according to their relative mobilities. *F*-statistics (Weir & Cockerham, 1984) were calculated for each locus to determine genetic structure among collections within species. The method by Weir & Cockerham (1984) gives an unbiased estimate of Wright's (1978) F_{ST}.

RESULTS

Genes presumed to be introgressed were found at one or two loci in seven out of ten sympatric

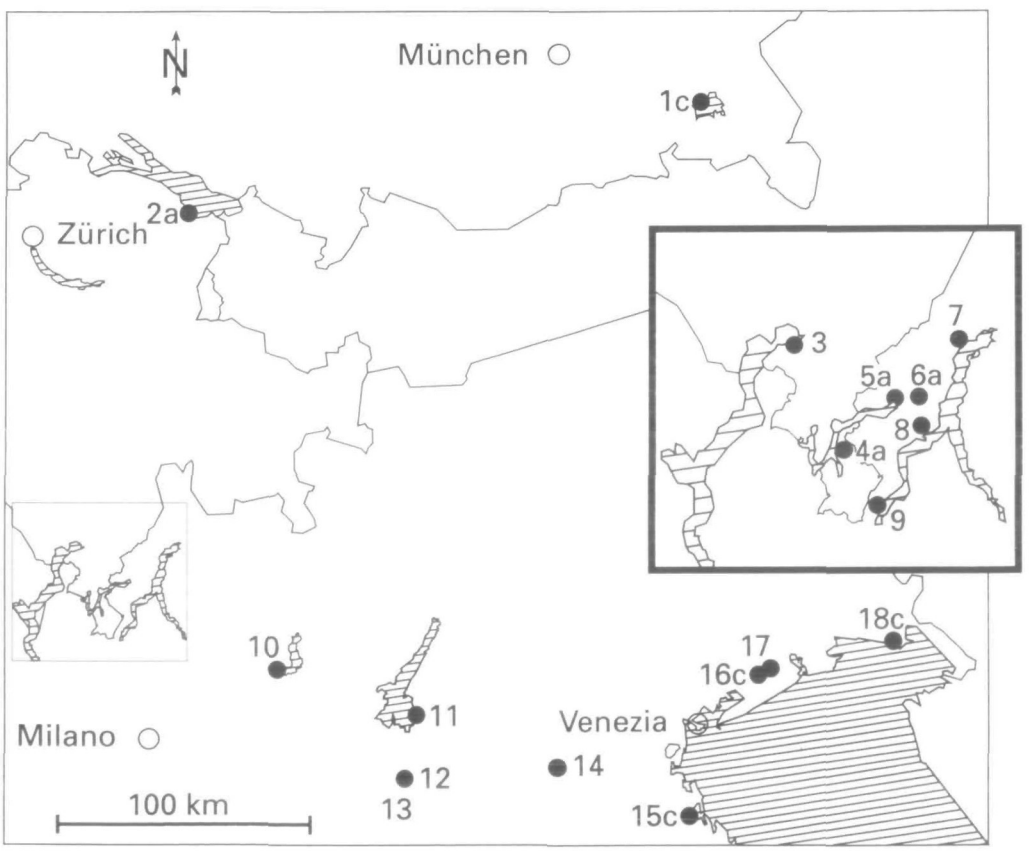

Figure 1. Collection sites for sympatric and allopatric populations of *Viviparus ater* and *Viviparus contectus* in Germany, Switzerland and Italy. Inset shows Lake Maggiore and Lake Como regions.

Table 1. Sampling sites and dates for sympatric and allopatric populations of *Viviparus ater* and *Viviparus contectus* in central Europe.

Code[1]	Location	Collection date	Collected species
1c	Lake Chiem, Germany	28/9/93	*V. conectus*
2a	Horn, Lake Constance, Switzerland	10/8/94	*V. ater*
3	Magadino, Lake Maggiore, Switzerland	7/8/94	*V. ater*
4a	Bissone, Lake Lugano, Italy	29/10/92	*V. ater*
5a	Porlezza, Lake Lugano, Italy	29/10/92	*V. ater*
6a	Lake Piano, Italy	29/10/92	*V. ater*
7	Gravedona, Lake Como, Italy	7/5/94	*V. ater* & *V. contectus*
8	Campo, Lake Como, Italy	6/5/94	*V. ater* & *V. contectus*
9	Cernobbio, Lake Como, Italy	9/5/94	*V. ater* & *V. contectus*
10	Sarnico, Lake Iseo, Italy	1 & 22/5/93	*V. ater* & *V. contectus*
11	Lake Garda, Italy from Katoh & Ribi (1996)		*V. ater* & *V. contectus*
12	East of Góito, Italy	2/5/93	*V. ater* & *V. contectus*
13	South of Góito, Italy	2/5/93	*V. ater* & *V. contectus*
14	Noventa, Italy	23/10/92	*V. ater* & *V. contectus*
15c	Rosolina, Italy	25/10/92	*V. contectus*
16c	Stretti 1, Italy	20/3/93	*V. contectus*
17	Stretti 2, Italy	21/3/93	*V. ater* & *V. contectus*
18c	Al Ponte, Grado-Fiumicello, Italy	22/3/93	*V. contectus*

[1]a and c indicate the existing species *Viviparus ater* and *Viviparus contectus*, respectively, at the allopatric sites.

Table 2. Allele frequencies of allopatric and sympatric populations of *Viviparus ater* from Switzerland and Italy. See Table 1 for names of the population sites.

Locus/ allele	Populations 2a	3	4a	5a	6a	7	8	9	10	11	12	13	14	17
βGAL														
**105*	–	–	–	–	–	–	0.05	–	–	0.002	–	–	0.09	–
**100*	0.99	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	0.998	1.00	1.00	0.86	1.00
**94*	0.01	–	–	–	–	–	–	–	–	0.000	–	–	0.05	–
GPI														
**119*	–	–	–	–	–	–	–	–	–	0.007	–	–	–	–
**100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.993	1.00	1.00	1.00	1.00
MDH-1														
**143*	–	–	–	–	–	–	0.01	–	–	–	–	–	–	–
**100*	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.997	1.00	1.00	1.00	1.00
**44*	–	–	–	–	–	–	–	–	–	0.003	–	–	–	–
PGM														
**100*	1.00	1.00	0.93	0.92	1.00	1.00	0.99	1.00	1.00	0.998	1.00	0.96	1.00	0.99
**93*	–	–	–	–	–	–	–	–	–	0.002	–	–	–	0.01
**82*	–	–	0.07	0.08	–	–	0.01	–	–	0.000	–	0.04	–	–
PGDH														
**148*	–	–	0.01	–	–	–	–	–	–	0.002	–	–	–	–
**100*	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.998	1.00	1.00	1.00	1.00
PNP														
**100*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.000	1.00	1.00	1.00	1.00
N	80	34	58	60	15	45	40	40	42	293	5	28	11	41

Frequencies of proposed introgressed alleles are underlined.
The most common allele in *V. ater* is designated as **100*; "–" means 0.00.
Data for Population 11 (Lake Garda, Italy) are from Katoh & Ribi (1996).

Table 3. Allele frequencies of allopatric and sympatric populations of *Viviparus contectus* from Germany and Italy. See Table 1 for names of the population sites.

Locus/ allele	Populations 1c	7	8	9	10	11	12	13	14	15c	16c	17	18c
βGAL													
**105*	1.00	0.99	1.00	0.89	1.00	0.995	1.00	1.00	1.00	1.00	1.00	1.00	1.00
**100*	–	–	–	0.11	–	0.005	–	–	–	–	–	–	–
**94*	–	0.01	–	–	–	0.000	–	–	–	–	–	–	–
GPI													
**144*	–	–	–	–	–	0.000	–	–	–	0.02	–	–	–
**119*	1.00	–	–	–	1.00	0.944	1.00	1.00	1.00	0.98	1.00	0.99	1.00
**100*	–	1.00	1.00	1.00	–	0.056	–	–	–	–	–	0.01	–
MDH-1													
**100*	–	0.01	–	–	–	0.008	–	–	–	–	–	–	–
**84*	–	–	–	–	–	0.001	–	–	–	–	–	–	–
**44*	1.00	0.99	1.00	1.00	1.00	0.991	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM													
**100*	–	–	–	–	0.06	0.022	–	–	–	–	–	–	–
**93*	0.10	–	0.09	0.09	0.10	0.031	–	0.11	0.40	0.74	0.60	0.54	0.61
**82*	0.90	1.00	0.91	0.91	0.84	0.947	1.00	0.89	0.60	0.26	0.40	0.45	0.39
**78*	–	–	–	–	–	0.000	–	–	–	–	–	0.01	–
PGDH													
**148*	1.00	–	–	–	0.65	0.470	1.00	1.00	0.85	0.62	0.33	0.50	0.05
**100*	–	1.00	1.00	1.00	0.35	0.530	–	–	0.15	0.38	0.67	0.50	0.95
PNP													
**122*	–	–	–	–	0.53	0.974	0.31	0.37	0.90	–	0.24	0.17	0.02
**100*	1.00	1.00	1.00	1.00	0.47	0.026	0.69	0.63	0.10	1.00	0.76	0.83	0.98
N	5	39	35	40	35	383	16	31	10	21	68	60	31

Frequencies of proposed introgressed alleles are underlined.
The most common allele in *V. ater* is designated as **100*; "–" means 0.00.
Data for Population 11 (Lake Garda, Italy) are from Katoh & Ribi (1996).

sites (*Viviparus ater* in Table 2 [Populations 8, 13, 14 and 17; *V. contectus* in Table 3 [Populations 7, 9, 10 and 17]). These seven sites covered most of northern Italy. Inferred directions of introgresson were from *V. contectus* to *V. ater* (Table 2) and vice versa (Table 3). However, two allopatric populations (Table 2; Populations 4a and 5a) of *V. ater* in Lake Lugano possessed *PGM*82* and *PGDH*148*, which were rather common alleles in *V. contectus* (Table 3), at low frequencies.

Viviparus ater had low mean heterozygosity (overall mean $H_e \pm$ S.D. = 1.1 ± 1.4%) and little genetic differentiation (Table 2). The jackknifed mean of Wright's $F_{ST} \pm$ S.E. over four loci was low (0.041 ± 0.004), which also indicates genetic homogeneity (Table 4). On the other hand, *V. contectus* showed high mean expected heterozygosity (overall mean $H_e \pm$ S.D. = 11.5 ± 7.6%) and high genetic differentiation especially at four loci (*GPI, PGM, PGDH,* and *PNP*; Table 3). The jackknifed mean of $F_{ST} \pm$ S.E. over six loci was very high (0.546 ± 0.166), which indicates high genetic differentiation among populations (Table 4). Sympatric populations of *V. contectus* in Lake Como, Italy (populations 7, 8 and 9) possessed only *GPI*100* and *PNP*100* at one pair of the linked loci (Table 3). *GPI* at the other sympatric and allopatric populations were nearly substituted by **119* (Table 3). Frequencies of *PNP*100*, which was a common allele in *V. ater* and only 2.6% in *V. contectus* in Lake Garda, were more than 50% in most populations of *V. contectus* in this study.

DISCUSSION

Presumably introgressed alleles were found from most sympatric populations of *Viviparus ater* and *V. contectus* in northern Italy. Some allopatric populations had genes which may have had their origin in introgression. But because shared alleles between species can be due to mutation or to common ancestry, the

Table 4. Hierarchical *F*-statistics for *Viviparus ater* and *Viviparus contectus* with jackknifed means and standard errors (S.E.).

Locus	F_{IS}	F_{ST}
Viviparus ater		
βGAL	0.476	0.046
MDH-1	0.001	−0.001
PGDH	0.005	−0.005
PGM	0.042	0.040
Mean	0.063	0.041
S.E.	0.213	0.004
Viviparus contectus		
βGAL	−0.099	0.082
GPI	−0.001	0.989
MDH-1	0.003	−0.003
PGDH	−0.134	0.492
PGM	0.115	0.329
PNP	0.080	0.296
Mean	0.029	0.546
S.E.	0.084	0.166

origin of each allele cannot be inferred from allele frequency data (Harrison, 1990). Therefore, we cannot rule out that observed patterns are due to shared ancestry. In fact, although *PNP*100* in *V. contectus* from Lake Garda was interpreted as introgressed allele in Katoh & Ribi (1996), the *PNP*100* in Lake Garda may be a simple polymorphic allele because *PNP* was highly variable at other populations in this study. However, *V. ater* and *V. contectus* can hybridize in nature and frequency of F_1 hybrids was 0.74% in Lake Garda (Katoh & Ribi, 1996). F_1 hybrids are viable and mainly males (Trüb, 1990; Katoh & Ribi, 1996). Females crossed with male F_1 hybrids produced back-crossed offspring (Trüb, 1990). Therefore, there is a possibility of widespread introgression or mosaic zones in nature. Moreover, the alleles *PGM*82* and *PGDH*148* of *V. ater* in Lake Lugano (allopatric sites) may have introgressed in the past when the two species coexisted.

Even if hybridization and introgression occur at many places, we do not expect that frequencies of introgressed alleles increase in time because *Viviparus ater* and *V. contectus* have reduced fitness of F_1 hybrids. In *Viviparus*, crosses between *V. ater* females and *V. contectus* males produced 50% of offspring of normal intraspecific *V. ater* fecundity (Trüb, 1990). The reverse cross produced only 2% of intraspecific *V. contectus* fecundity. The fecundity of the back-crossed snails was about half of normal conspecific matings. In spite of these disadvantages, these species could maintain a low level of gene leakage through interspecific and back-cross matings.

The six allozyme loci indicated high genetic differentiation in *Viviparus contectus* and low genetic differentiation in *Viviparus ater*. The presence of many isolated small populations seems to cause high differentiation in *V. contectus*. *V. contectus* prefers small streams and swamps where populations tend to be small and *V. ater* lives primarily in larger bodies of water such as lakes and rivers where populations are large (Franz, 1932; personal observation). Other brooding *Viviparus* species in the United States show large genetic differentiation among and within drainage systems (Katoh & Foltz, 1994). In general, freshwater gastropod populations tend to be genetically differentiated (Brown & Richardson, 1988; Jarne & Delay, 1991). In addition, the low genetic variation found in *V. ater* based on 13 allozyme loci suggests a severe bottleneck event in the past (Porter & Ribi, 1994). Although low introgression may have happened several times at many places where *V. ater* and *V. contectus* coexisted, the genetic differences which were observed between *V. ater* and *V. contectus* in this study can be explained by the presence of many isolated small populations of *V. contectus* and a past bottleneck event in *V. ater*.

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